Nucleotide C3′,4′-Radical Cations and the Effect of a 2′-Oxygen Substituent. The DNA/RNA Paradox

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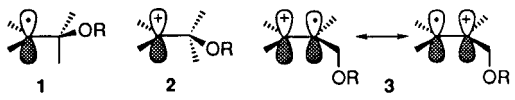
The chemistry of carbon-centered free radicals is only perturbed to a minor extent by the presence of β-hydroxy and β-alkoxy groups. The β-ethoxyethyl radical (**1**) preferentially adopts a staggered conformation with the C−O bond synclinal to the axis of the half-filled p-orbital.[1] β-Alkoxy groups do not accelerate the stannyl radical induced fragmentation of thiocarbonyl esters in fully conformationally labile systems,[2] and that sometimes observed in carbohydrates and in some more rigid cases[2,3] is best attributed to the relief of unfavorable steric and/or dipolar interactions.[2] However, decelerations are observed for hydrogen atom abstractions vicinal to alkoxy groups by electrophilic radicals owing to the polar nature of the transition state which places a δ^+ charge β to the C−O bond.[4−6] A minor retardation of hydrogen transfer from thiols to alkyl radicals is occasioned by vicinal alkoxy groups, again due to the polar nature of the transition state.[7] Conversely, the chemistry of the corresponding carbocations (**2**)[8] is very significantly affected by the alkoxy group. Thus, Jencks and



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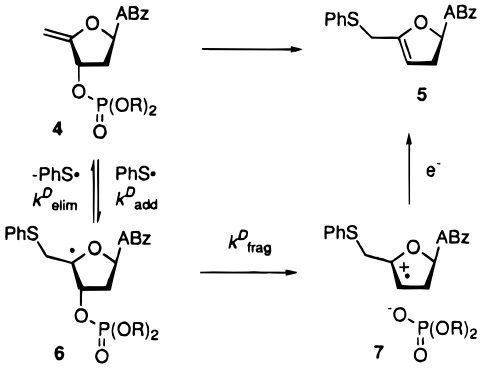
Amyes demonstrated that 2-azido-1,2-dimethoxypropane undergoes solvolysis in aqueous solution some 600 times less rapidly than 2-azido-2-methoxypropane.[9] Probably, the most widely appreciated manifestation of this effect is the relative instability of 2-deoxyglycopyranosides when compared to simple methyl glucopyranoside in aqueous acid, wherein a 2000-fold difference in rate of hydrolysis is observed.[10] We asked, "how do alkoxy and/or hydroxy groups affect the chemistry of the related radical cations (**3**), especially their rate of formation by fragmentation of β-(phosphatoxy)alkyl radicals?" In this paper, we report our preliminary findings in this area and discuss possible implications.

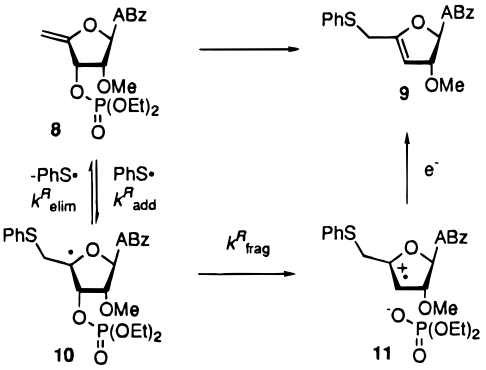
In his study of the chemistry of nucleotide C4′-based radicals,[11] Giese described the reaction of the *exo*-glycal **4a** with thiophenol leading to the formation of the allylic substitution product **5**.[12] This reaction, which did not proceed in the absence of light or radical initiator, was rationalized by addition of the PhS• radical to **4a** to give the C4′ radical **6a** which then underwent fragmentation to the radical cation **7**. Electron transfer from thiol or thiolate finally yields **5** (Scheme 1). Subsequent work with related C4′ radicals put such fragmentations beyond doubt and firmly established the formation of radical cations as trapable intermediates.[13,14]

Scheme 1



a: R = Ph; **b:** R = Et. ABz = 6-*N*-benzoyladenine

Scheme 2



ABz = 6-*N*-benzoyladenine

We prepared the corresponding diethyl phosphate **4b** and the 2′-*ribo*-methoxy analog **8** by standard means. The deoxynucleotide **4b** (0.1 M) was treated with 30 equiv of thiophenol in CD_3OD/D_2O (10:1) and stirred at 40 °C in the presence of di-*tert*-butyl peroxalate (50 mol %) as radical initiator, leading to completion within 10 min and isolation of **5** in 87% yield (Scheme 1). The large amount of initiator required is suggestive of a nonchain process and is fully consonant with the suggested mechanism. However, under the same conditions, **8** was essentially unchanged, despite repeated addition of initiator, after 24 h (96% recovery, Scheme 2). That the dramatic difference in rate was a factor of the 2′-methoxy group, and not of some unidentified radical inhibitor in **8**, was established by the reaction of an ∼1:1 mixture of **4b** and **8** with thiophenol under otherwise identical conditions: Figure 1 is a plot of the ratio of **8**:**4b** with time with addition of 10 mol % of initiator every 0.5 h until 76% consumption (2 h) of **4b**. This retarding effect of the methoxy group, however, may be compensated by the use of a better leaving group. Under typical conditions, the diphenyl phosphate **12** gave **9** in 88% isolated yield after 10 min.

In interpreting these results, we assume that the rate constants for addition of PhS• to **4b** (k^D_{add}) and to **8** (k^R_{add}) are essentially identical, as are those for the corresponding reverse reactions (k^D_{elim} and k^R_{elim}), i.e., that a γ-methoxy substituent has no

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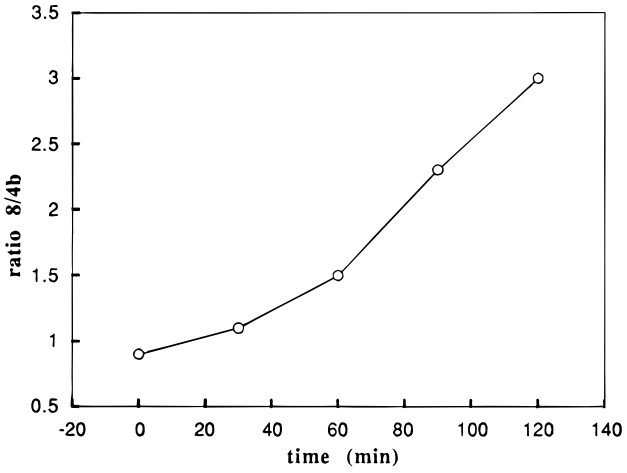
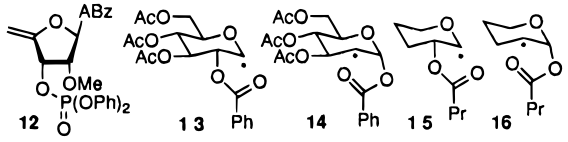


Figure 1.

significant effect on the stability of a carbon radical. This assumption is justifiable on the grounds that simple γ-hydroxy- and -alkoxypropyl radicals have been shown by ESR spectroscopy to be sp^2-hybridized and non-bridging in aqueous and organic solutions.[15−18] Nevertheless, recent work by Jenkins demonstrated that γ-alkoxy groups very slightly accelerate (<2-fold) the abstraction of hydrogen atoms in organic solution by *tert*-butoxy radicals, suggestive of some bridging.[5,6] However, this effect is so small as to be insignificant in the present context. The difference in reactivity of **4b** and **8** can be attributed therefore to a very significant difference in the rate constants (k^{D}_{frag} and k^{R}_{frag}) for fragmentation of the C4′ radicals. This is readily understood in terms of the destabilization of **11** and of the preceding, polarized transition state, by the inductively electron-withdrawing methoxy group.

This observation has ramifications in several fields. The antitumor antibiotic iron·bleomycin (BLM) efficiently cleaves DNA by generation of C4′ nucleotide radicals[19−21] but is much less efficient, and more sequence selective, in the parallel degradation of RNA.[22,23] Experiments by the Hecht group show that BLM binds to $tRNA^{His}$ more tightly than to the corresponding "$tDNA^{His}$", yet the tDNA was degraded at lower concentrations of BLM.[24] These observations prompted the suggestion that either BLM binds to RNA "in an orientation not conducive to the initiation of a subsequent chemical event that leads to alteration of RNA structure" or, alternatively, that "the chemical alteration of RNA by BLM may lead in part to one or more products that do not involve strand scission".[24] If[19] the Giese mechanism[11] is applicable to the cleavage of oligonucleotides by BLM, then, at least under relatively hypoxic conditions, the present results indicate that the apparent reduced reactivity of RNA will be, at least in part, a function of the longer lifetime of its C4′ radicals which will permit repair by endogenous thiols and/or favor cleavage of the C5′−O bond. The β-(acyloxy)-



alkyl[25] migration **13** → **14** occurs with complete inversion of the carbonyl and carboxyl oxygens[26] suggestive of a 5-center/5-electron concerted shift, yet that of the related **15** → **16**, in addition to having substantially different kinetic parameters, takes place with significant scrambling of the two oxygens,[27] indicating that a radical cation/carboxylate anion pair is responsible, at least in part, for this rearrangement. This dichotomy is now readily seen to be a function of the C3-acyloxy group in **13** which significantly retards fragmentation to the radical cation and thus favors the concerted shift.

Further work on the chemistry of heteroatom-substituted radical cations is currently underway.

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Supporting Information Available: Preparation and listings of characterization data for **4b**, **5**, **8**, **9**, and **12**, and experimental details for the fragmentation reactions (10 pages). See any current masthead page for ordering and Internet access instructions.

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